REMARKS

By this amendment, claims 1, 3, 4, 5 and 9 have been amended, claims 10 and 11 have been added, and claims 2 and 8 have been cancelled without prejudice. Claims 1, 3-7, and 9-11 are thus currently under examination in the present application. For the reasons set forth below, Applicants submit that the present amendments and arguments place this application in condition for immediate allowance.

As an initial matter, with regard to new claims 10 and 11, these claims have been added to incorporate alternative dependencies that were initially presented in claim 5 of the application, as filed. Accordingly, no new matter has been added by these amendments.

In the Office Action of June 24, 2008, the Examiner first objected to claim 4 for not having a period at the end of the claim. This objection has now been rendered moot by the present amendment which inserts a period at the end of claim 4, and thus the objection should be withdrawn.

In the Office Action, the Examiner then made various rejections to claims 1-9 under 35 U.S.C. §112, second paragraph as being indefinite. In particular, the Examiner asserted that certain terms and phrases in claims 1, 2, and 9 were unclear. Each of these rejections is addressed in detail below and, accordingly, for the reasons set forth below, Applicants submit that the Examiner's rejections are respectfully traversed and should be withdrawn.

With regard to the Examiner's rejection of claim 1 under 35 U.S.C. §112, second paragraph, the Examiner has asserted that the complete structure of the hapten is unclear in terms of both the moieties attached to the hapten and the linkage position of the carrier protein, and, further, that it is unclear how the CH3O-C(CH3)2-CH2 moiety is attached to the carrier molecule. These assertions have now become moot by virtue of the present amendments to claim 1. Specifically, by virtue of the present amendments, Applicants have amended claim 1 to incorporate the limitation previously found in claim 2 indicating that the "hapten is: CH3O-C(CH3)2-CH2-X-B where X is a spacer and B is a group capable of binding to a carrier protein..." As such, claim 1 now provides a complete structure of the hapten and thus clearly indicates the particular moieties that comprise the hapten molecule as well as the linkage position of the carrier protein. Further, by the present amendments to claim 1, "B" is indicated as "a group capable of binding to a carrier protein" and thus particularly points out how the hapten is conjugated to a carrier protein.

With regard to the Examiner's rejection of claim 2 under 35 U.S.C. §112, second paragraph, the Examiner asserted that the terms "spacer" and "group capable of binding to a carrier protein" were indefinite and thus it was unclear what compounds or groups were encompassed by these terms. By the present amendments, these terms no longer appear in claim 2 due to the cancellation of claim 2, but are instead are recited in claim 1. Nevertheless, contrary to the Examiner's assertions, both of these terms are routinely used in the art to identify various mechanisms for suitably conjugating a portion of a hapten and, thus comply with the requirements of 35 U.S.C. §112, second paragraph.

Indeed, a quick database search of the phrase "hapten conjugation spacer" uncovered over 18,000 references showing that the term "spacer" is widely used in the art in relation to haptens. For example, the attached abstract of Yoo, et al., which was uncovered in the search, recites that "five haptens of fenthion differing in spacer arm length (4-8 atoms) were synthesized and...conjugated to bovine serum albumin and keyhole limpet hemocyanin to be used as immunogens" Further, the attached abstract of Ungar-Waron is directed toward the "Role of a rigid polyproline spacer inserted between hapten carrier in the induction of anti-hapten antibodies and delayed hypersensitivity." As yet another example of the use of the term "spacer" when referring to haptens, the attached abstract of "Shinkaruk, et al., which was also uncovered in the search discussed above, begins with "[t]wo carboxylic acid haptens of glycitein were synthesized, with a spacer arm at the C2 position. They differed in the length of the spacer arm, with the length of the spacer arms being three or four carbon atoms" and further recites that "..specificity is not determined by the length of the spacer arm."

Furthermore, U.S. Patent No. 5,219,764 to Huber, et al. refers in its abstract to "Hapten-biotin conjugates in which the hapten is linked with biotin via a spacer, which has 26 to 40 atoms in its chain..." It is further noted that claim 1 of the Huber, et al. reference includes precisely the same wording, without any limitations on what is meant by the term "spacer." Indeed, column 3, lines 19-24 of the Huber, et al. reference merely indicates that "[t]he production of the conjugates...can either take place by reacting the hapten and the biotin with a bi-functional spacer molecule in which functional groups present on the hapten and in the biotin molecule react with the functional groups of the

spacer molecule." As one of ordinary skill in the art would further recognize, these general references to "functional groups of the spacer molecule" are precisely what is being referred to in the present application by the use of the phrase "group capable of binding to a carrier protein."

Accordingly, in light of the foregoing discussion of the use of the term "spacer" and the phrase "group capable of binding to a carrier protein" in the various references cited above, it is thus clear that these terms are routinely used in the art when referring to haptens and thus create no indefinite issues whatsoever.

With regard to the Examiner's rejection of claim 9 under 35 U.S.C. §112, second paragraph, the Examiner asserted that claim 9 is indefinite because it does not recite any positive steps for a method of assaying a sample for fuel oxygenates. Contrary to the Examiner's assertions, however, claim 9 is a proper method claim which recites positive steps. Claim 9 is directed to a method for assaying a sample for fuel oxygenates that not only comprises generating antibodies according to the method of claim 1, but further comprises the step of carrying out an immunoassay using the antibodies. As such, claim 9 recites a method which is comprised of at least eight steps, namely the seven steps recited in claim 1 together with the step of "carrying out an immunoassay using said antibodies" and thus cannot be fairly characterized as being indefinite for reciting a method that does not set forth any steps. In this regard, it is also further noted that the phrase "an immunoassay using antibodies" is well known in the art, and thus it would not be necessary to further clarify the additional steps that are involved in carrying out such an immunoassay.

In any event, in order to eliminate any objection on this point, Applicants have amended Claim 9 so that the word "and" has been replaced by "further comprising" which Applicants submit has the same meaning and scope of the prior language.

In light of the discussion above, Applicants thus submit that the claims of the present application are in compliance with 35 U.S.C. §112, second paragraph. Accordingly, Applicants respectfully submit that the Examiner's rejection under 35 U.S.C. §112, second paragraph is respectfully traversed and should be withdrawn.

In the Office Action of June 24, 2008, the Examiner further rejected claim 9 under 35 U.S.C. §101 because the claimed recitation of a use, without setting forth any steps involved in the process, was an improper definition of a process. As discussed above, however, in relation to the Examiner rejection of claim 9 pursuant to 35 U.S.C. §112, second paragraph, Claim 9 recites a method which is comprised of at least eight steps, the seven steps recited in claim 1 together with the step of "carrying out an immunoassay using said antibodies." Further, Applicants have amended Claim 9 so that the word "and" has been replaced by "further comprising". As such, Applicants respectfully submit that claim 9 is a proper process claim under 35 U.S.C. §101 and, consequently, that the Examiner's rejection is respectfully traversed and should be withdrawn.

Finally, in the Office Action, the Examiner rejected claim 8 under 35 U.S.C. §102(e) as being anticipated by or, in the alternative, under 35 U.S.C. §103 as being obvious over Pourfarzaneh (U.S. Pat. No. 6,416,671). Without addressing the merits of this rejection, this rejection has now become moot by virtue of the present amendments cancelling claim 8 and, accordingly, the Examiner's rejection should be withdrawn.

In light of the amendments and arguments provided herewith, Applicants submit that the present application overcomes all prior rejections and objections, and has been placed in condition for allowance. Such action is respectfully requested.

Respectfully submitted,

Date: February 2, 2008

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Article

Role of a rigid polyproline spacer inserted between hapten and carrier in the induction of anti-hapten antibodies and delayed hypersensitivity

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ABSTRACT

The synthesis and characterization are described of a hapten-protein conjugate in which carrier [BSA or the synthetic multichain polymer, poly-L-(Tyr, Glu)-poly-DL-Ala-poly-LLys, (T,G)-A-L] and hapten 2,4-dinitrophenyl (DNP) are separated by the rigid peptide chain of an L-proline oligomer. This compound has been used to study the carrier effect in an attempt to distinguish between the cooperation and local environment theories which have been put forward to explain it.

Rabbits were given a primary injection of the antigen DNP-poly-Lprolyl-BSA (bovine serum albumin). The secondary response was followed *in vitro* after stimulation with antigens composed of hapten coupled to carriers in the presence or absence of a polyproline spacer, and compared to secondary stimulation with the homologous antigen. Antibody production was monitored by thymidine incorporation, by inactivation of modified bacteriophage and by hemolytic plaque formation. Experiments in delayed hypersensitivity were also carried out using the same antigens. In both systems it was found that the insertion of the inflexible spacer molecule between hapten and carrier did not abolish the carrier effect. These results lend support to the cooperation hypothesis as explanation of the carrier effect.

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1: J Agric Food Chem. 2008 Aug 27;56(16):6809-17. Epub 2008 Jul 23.

Synthesis of haptens and conjugates for ELISA of glycitein: development and validation of an immunological test.

Shinkaruk S, Lamothe V, Schmitter JM, Fructus A, Sauvant P, Vergne S, Degueil M, Babin P, Bennetau B, Bennetau-Pelissero C.

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Two carboxylic acid haptens of glycitein were synthesized, with a spacer arm at the C2 position. They differed in the length of the spacer arm, with the length of the spacer arms being three or four carbon atoms, and were named Delta3-glycitein and Delta4-glycitein haptens, respectively. The different haptens were coupled to bovine serum albumin (BSA), and the coupling efficiency was assessed by MALDI mass spectrometry. Polyclonal antibodies were generated against the BSA conjugates. An additional conjugate of Delta4-glycitein hapten was generated with swine thyroglobulin (Thyr). Enzyme-linked immunosorbent assays (ELISAs) based on the competition between free glycitein and Delta4-glycitein-Thyr conjugates for specific antibodies were developed. The IC50 of the standard curves was 15.6 ng mL(-1) with anti-Delta3-glycitein and 62.5 ng mL(-1) with anti-Delta4-glycitein, that is, 10.9 and 44 pmol/well, respectively. With the Delta3-glycitein antibody, interassay and intra-assay variations were 12.2 and 11.5%, respectively. Specificity tests did not show any significant cross-reaction with any other soy isoflavone. This specificity is not influenced by the length of the spacer arm. The assay was validated by measurements performed on plasma samples as well as on soy-based foodstuffs and on soy-based food supplements.

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Titre du document / Document title
Synthesis of haptens for immunoassay of organophosphorus pesticides and effect of heterology in hapten spacer arm length on immunoassay sensitivity

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Résumé / Abstract
The synthetic method for haptens of organophosphorus (OP) pesticides with a spacer arm (amino carboxylic acid) attached at the pesticide thiophosphate group was simplified to a large extent. While the previous synthetic approach for this type of haptens requires seven steps, the present process involves only two steps. Using this process, five haptens of fenthion differing in spacer arm length (4-8 atoms) were synthesized and they were conjugated to bovine serum albumin and keyhole limpet hemocyanin to be used as immunogens. Rabbits were immunized with these hapten-protein conjugates for production of polyclonal antibodies against fenthion. The five haptens were conjugated to ovalbumin to be used as plate-coating antigens and twenty polyclonal antisera to the haptens were screened against each of the five coating antigens using noncompetitive and competitive indirect enzyme-linked immunosorbent assay (ELISA). The titer difference between the homologous and heterologous combinations was small, suggesting that heterology in spacer arm length is not important for the antigen recognition by antibodies. While the heterology in spacer length of the coating antigen had no significant effect on the sensitivity of ELISA, heterology in spacer structure of the coating antigen produced a remarkable improvement in the sensitivity of ELISA.

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Huber et al.

[11] Patent Number: 5,219,764

[45] Date of Patent: Jun. 15, 1993

[54] HAPTEN-BIOTIN CONJUGATES AND THEIR USE

[75] Inventors: Erasmus Huber, Unterfinning; Dietmar Zdunek, Munich; Christian Klein; Roland Schenk, both of Weilheim, all of Fed. Rep. of Germany

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[21] Appl. No.: 683,284

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Assistant Examiner—Lora M. Green
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[57] ABSTRACT

Hapten-biotin conjugates in which the hapten is linked with biotin via a spacer, which has 26 to 40 atoms in its chain and contains at least 5 heteroatoms, are novel and are suitable, in particular for use in a competitive homogeneous immunoassay in which the agglutination which occurs in the reaction is evaluated by turbidimetric or nephelometric measurements.

6 Claims, No Drawings